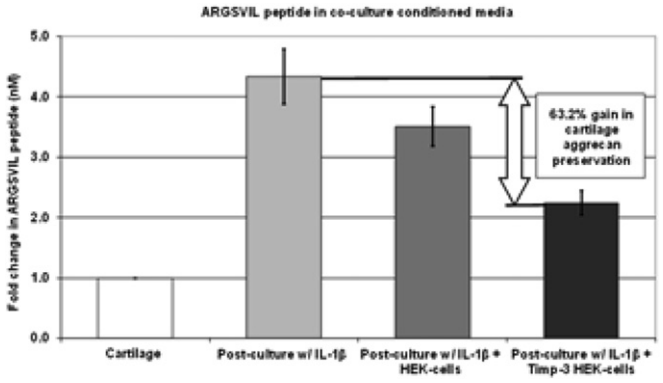

Figure 3. Aggrecan changes in articular cartilage exposed to TIMP-3. Co-culture with TIMP-3-transduced cells significantly decreased the amount of the degraded -aggrecan fragment, ARGSVIL released into the media of IL-1β-cultured cartilage. Error bars represent SE.

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CHANGES ON THE EXPRESSION OF CD44 IN IMMOBILIZED KNEE IN RATS

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Purpose: Both acute and chronic high-intensity loads on the knee joint caused cartilage degeneration, which may eventually lead to osteoarthritis. Likewise, joint immobilization following spinal cord injuries or secondary to treatments for acute musculoskeletal injury could also result in catabolic responses on articular cartilage. Previous studies have demonstrated that immobilization of the knee joint caused cartilage thinning, tissue softening and reduced proteoglycan content in vivo. Turnover of matrix hyaluronan and the hyaluronan binding region of aggrecan is mediated by CD44. The capacity for cell signaling by CD44 exhibits cell-type specificity, and those cascades in chondrocyte have not yet been defined clearly. In this study, we examined the expression of CD 44, which could be the indicative of the chondrocyte metabolism and the histological changes of the cartilage in the immobilized knee in rats.
Methods: The unilateral knee joint of Wistar rats aged 8-week old were immobilized at 140±5 degrees of knee flexion with metal screws, wire and resin for 1,2,4,8 or 16 weeks. Sham operated had holes drilled in the femur and tibia with screws, but the joint motion was not restricted. The immobilized operation and sham-operation made up the immobilized group. A control group had no surgery on their knees. Forty rats were prepared for histological analysis (immobilized group: n=5/each period, control group: n=3/each group). Also we identified the loss of extension of the range of motion (ROM) at the immobilized knee joint. Rats were sacrificed at 1, 2, 4, 8 and 16 weeks after the operation. The knees were removed, fixed with 4% PFA, decalcified in 10% EDTA and embedded in paraffin. Six-μm sections were obtained at the medial mid-condylar region of the knee in the sagittal plane. The sections were stained with hematoxylin-eosin. In the two areas (non-contact and contact) from the articular surface of femur and tibia, the number of chondrocytes was counted and thickness of total articular cartilage of each area was measured. The sections were also stained with rabbit anti-rat CD44 antibody (Abcam, ab65829) and the number of CD44(+) cells per region was counted.
Results: There was a progressive loss of extension ROM in the immobilized knee joint with time in immobilized groups. In histology, in the non-contact area, no significant change in the cartilage thickness was observed in control and immobilized group. The cartilage thickness in the contact area was gradually decreased throughout the experimental period in the immobilized group especially at tibia region. The number of chondrocytes in the contact area changed significantly throughout the experimental periods after immobilization especially at 16 weeks in the tibia region. There was not a significant change in the non-contact area. In control group, CD44 expression has been observed in whole area of cartilage. In immobilized group, Depth-dependent less expression pattern of CD44 was observed with time after immobilization, especially in 8 and 16 weeks. This pattern observed both of areas, non-contact and contact in immobilized groups. The less expression pattern was observed near the calcified cartilage and subchondral bone. The ratio of CD44 positive chondrocytes was gradually decreased in immobilized group, especially contact area.
Conclusions: CD44 participates in the internalization and turnover of HA in articular chondrocytes. Current results indicated that disruption of the HA-CD44 interaction occurred and may disturb cartilage homeostasis at the cartilage of immobilized joint. Furthermore, these change occur deeper layer than superficial layer at the cartilage.

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PLATELET RICH PLASMA FROM DIFFERENT PREPARATIONS: EFFECT ON CHONDROCYTES AND SYNOVIOCYTES OF OSTEOARTHRITIS PATIENTS

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Purpose: Platelet Rich Plasma (PRP) is an autologous blood derived product used as promising intra-articular treatment for cartilage degenerative lesions in osteoarthritis (OA). However, the results on the potentiality of this biological treatment are still controversial and confounding, due to the lack of well-designed studies and to the different preparation procedures.
Aim of this study was to increase knowledge concerning the biological effect of PRP treatment on clinically relevant joint target cells. For this purpose, we compared two different PRP preparations on the expression of a panel of biomolecules by OA cultured synoviocytes and chondrocytes.
Methods: Blood collected from 7 volunteers was used to obtain the two PRP preparations, according to Anitua E. et al, (J Periodontal 2008)(PRP-1) and according to Filardo G. et al, (Knee Surg Sports Traumatol Arthrosc 2012) (PRP-2). Cartilage and synovial tissues were collected from OA patients (Kellgren-Lawrence grade I-III) undergoing knee replacement surgery. Isolated chondrocytes and synoviocytes were cultured in appropriate medium, supplemented with 5%, 10% and 20% PRP-1, PRP-2 and Poor Platelet Plasma (PPP).
Alamar Blue test was performed at day 0, 3 and 6 to test cell viability and proliferation.
Gene expression analysis was performed at day 7 by semi-quantitative RT-PCR. IL-1β, IL-6, IL-8, TNF, MMP-13, TIMP-1, VEGF, TGFb1, IL-10, FGF-2, HGF, Hyaluronic acid synthases (HAS)-1, -2, -3 were evaluated on both cell types; TIMP-3, -4, IL-4,-13 on synoviocytes only and Coll-II, SOX-9, aggrecan on chondrocytes only. Hyaluronic acid (HA) production was analyzed in culture supernatants by ELISA. PRP treated samples were compared to PPP ones at the same concentration and all results were expressed as relative increments.
Statistical analysis was carried out using the GraphPad Prism for Windows.
Results: a) PRP-2 white blood and platelets concentrations were significantly higher than PRP-1 concentrations ($p<0.001$ and $p<0.01$ respectively).
b) Proliferation and cell viability detected by Alamar Blue was similar in the different culture conditions.
c) HA production and HAS-2 gene expression were significantly enhanced by 20%PRP-2 compared to 20%PRP-1, both in syoviocytes and chondrocytes.
d) IL-1β, IL-8, TIMP-1, FGF-2 and VEGF were significantly induced by PRP-2 (10% and 20% PRP-2 vs PRP-1 $p<0.05$) only in synoviocytes. Conversely, PRP-1 stimulated the expression of TIMP-3, TIMP-4 and HGF ($p<0.05$).
Conclusions: The primary findings of this study suggest that the two PRP preparations differently induced the expression of several factors on clinically relevant target cells 'in vitro', this could be related to the amount and type of PRP cellular components.

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THE EFFECTS OF THERAPEUTIC EXERCISE ON THE BALANCE OF WOMEN WITH KNEE OSTEOARTHRITIS: A SYSTEMATIC REVIEW

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Purpose: The objective of this review was to examine evidence regarding the effects of therapeutic exercise on the balance of women with knee osteoarthritis (OA).